Claims

1. Process for generating and detecting recombinant DNA sequences in Saccharomyces cerevisiae comprising the steps of:

a) generating first diploid S. cerevisiae cells bearing in a defined locus of their genome a first recombination cassette comprising a first DNA sequence to be recombined, which is flanked by at least a first and a second marker sequences, and **in an** allelic position a second recombination cassette comprising a second DNA sequence to be recombined, which is flanked by at least a third and a fourth marker sequences,

b) inducing the sporulation of the first diploid cells obtained in a) and

c) isolating haploid cells containing recombination cassettes in which first recombined DNA sequences are flanked by at least the first and fourth marker sequences, and haploid cells containing recombination cassettes in which second recombined DNA sequences are flanked by at least the second and the third marker sequences.

2. Process according to claim 1, comprising further the steps of:

a) generating second diploid cells by mating haploid cells containing the first recombined DNA sequences obtained in 1c) with haploid cells containing second recombined DNA sequences obtained in 1c),

b) inducing the sporulation of the second diploid cells obtained in a) and

c) isolating haploid cells containing recombination cassettes in which third recombined DNA sequences are flanked by at least the first and the second marker sequences, and haploid cells containing fourth recombination cassettes in which fourth recombined DNA sequences are flanked by at least the third and the fourth marker sequences.

3. Process according to claim 1 or 2, wherein further recombined DNA sequences are generated by subjecting the haploid cells obtained in 2c) at least once to another cycle of mating with other haploid cells, inducing the sporulation of the diploid cells obtained and isolating haploid cells with recombined DNA sequences on the basis of the molecular linkage between two marker sequences.

4. Process according to any one of claims 1 to 3, wherein the first diploid cell is generated by simultaneously or sequentially transforming a diploid S. cerevisiae cell with a DNA molecule containing the first recombination cassette and a DNA molecule containing the second recombination cassette and optionally allowing the integration of the two recombination cassettes into allelic positions of the S. cerevisiae genome.

5. Process according to claim 4, wherein the DNA molecule comprising the first or the second recombination cassettes is a yeast artificial chromosome (YAC).

6. Process according to claim 4, wherein the DNA molecule comprising the first or the second recombination cassettes is a cloning vehicle, whereby the respective two marker sequences are flanked by targeting sequences which are homologous to a defined locus of the S. cerevisiae genome.

7. Process according to according to any one of claims 1 to 3, wherein the first diploid cell is generated by fusing a haploid S. cerevisiae cell bearing in a locus of its genome the first recombination cassette with a haploid S. cerevisiae cell bearing in an allelic position the second recombination cassette.

8. Process according to according to any one of claims 1 to 3, wherein the first diploid cell is generated by mating a haploid S. cerevisiae cell bearing in a locus of its genome the first recombination cassette with a haploid S. cerevisiae cell bearing in an allelic position the second recombination cassette.

9. Process according to claim 7 or 8, wherein haploid cells bearing the first or second recombination cassette are generated by:

a) inserting the first DNA sequence to be recombined between the first and the second marker sequences located adjacently on a first cloning vehicle and inserting the second DNA sequence to be recombined between the third and the fourth marker sequences located adjacently on a second cloning vehicle, whereby the respective two marker sequences are flanked by targeting sequences which are homologous to a defined locus of the S. cerevisiae genome,

b) excising from the cloning vehicles obtained in a) fragments bearing the first recombination cassette and the second recombination cassette, respectively, whereby each of the cassettes comprises the DNA sequence to be recombined flanked by the respective two marker sequences, and each cassette in turn is flanked by targeting sequences,

c) transforming the fragments bearing the recombination cassettes with flanking targeting sequences obtained in b) separately into S. cerevisiae diploid cells, whereby the targeting sequences direct the integration of the cassettes into that locus to which they are homologous, in order to obtain diploid cells heterozygous for the first cassette, or the second cassette,

d) inducing separately the sporulation of the heterozygous diploid cells obtained in c) and

e) isolating haploid cells containing the first cassette and expressing the first and second marker sequences and separately haploid cells containing the second cassette and expressing the third and the fourth marker sequences.

10. Process according to claim 9, wherein the first cloning vehicle is plasmid pMXY9 and the second cloning vehicle is plasmid pMXY12.

11. Process according to any one of claims 4-6, 9 or 10, wherein the diploid S. cerevisiae cells used for transformation are auxotrophic for at least two nutritional factors.

12. Process according to claim 11, wherein the diploid cells are homozygous for the *ura3-1* allele and the *trp1-1* allele, which render them auxotrophic for uracil and tryptophan, respectively.

13. Process according to any one of claims 4-6 or 9-12, wherein the diploid cells used for transformation are resistant to at least two antibiotics.

14. Process according to claim 13, wherein the diploid cells are homozygous for the *can1-100* allele and the *cyh2R* allele, which render them resistant to canavanine and cycloheximide, respectively.

15. Process according to any one of claims 4-6 or 9-14, wherein diploid cells of the S. cerevisiae strain MXY47 are used for transformation, which are homozygous for the alleles *ura3-1*, *trp1-1*, *can1-100* and *cyh2R* and heterozygous for the *msh2::KanMX* mutation.

16. Process according to any one of claims 1 to 15, wherein the S. cerevisiae cells have a functional mismatch repair system.

17. Process according to any one of claims 1 to 15, wherein the S. cerevisiae cells are transiently or permanently deficient in the mismatch repair system.

18. Process according to claim 17, wherein the transient or permanent deficiency of the mismatch repair system is due to an mutation and/or an inducible expression or repression of one or more genes involved in the mismatch repair system, a treatment with an agent that saturates the mismatch repair system and/or a treatment with an agent that globally impairs the mismatch repair.

19. Process according to any one of claims 1 to 18, wherein the first and the second recombination cassettes are integrated in the *BUD31-HCM1* locus on chromosome III of the S. cerevisiae genome.

20. Process according to any one of claims 1 to 19, wherein the first and the second DNA sequences to be recombined diverge by at least 1 nucleotide.

21. Process according to any one of claims 1 to 20, wherein the first and the second DNA sequences to be recombined are derived from organisms other than and including S. cerevisiae.

22. Process according to any one of claims 1 to 21, wherein the first and the second DNA sequences to be recombined comprise one or more non-coding sequences and/or one or more protein-coding sequences.

23. Process according to any of claims 1 to 22, wherein the marker sequences are selected from the group consisting of nutritional markers, pigment markers, antibiotic resistance markers, antibiotic sensitivity markers, primer recognition sites, intron/exon boundaries, sequences encoding a particular subunit of an enzyme, promoter sequences, downstream regulated gene sequences and restriction enzyme sites.

24. Process according to claim 23, wherein the first and third marker sequences are nutritional markers, the gene products of which can compensate an auxotrophy of a S. cerevisiae cell.

25. Process according to claim 24, wherein the first marker sequence is *URA3*, the gene product of which can confer uracil prototrophy to an uracil auxotrophic S. cerevisiae cell.

26. Process according to claim 24, wherein the third marker sequence is *TRP1*, the gene product of which can confer tryptophan prototrophy to an tryptophan auxotrophic S. cerevisiae cell.

27. Process according to claim 23, wherein the second and fourth marker sequences are antibiotic sensitivity markers, the gene products of which can confer sensitivity to an antibiotic to a S. cerevisiae cell which is resistant to that antibiotic.

28. Process according to claim 27, wherein the second marker sequence is *CAN1*, the gene product of which can confer sensitivity to canavanine to a canavanine-resistant S. cerevisiae cell.

29. Process according to claim 27, wherein the fourth marker sequence is *CYH2*, the gene product of which can confer sensitivity to cycloheximide to a cycloheximide-resistant S. cerevisiae cell.

30. Process according to any one of claims 1 to 29, wherein haploid cells containing recombination cassettes with either first, second, third or fourth recombined DNA sequences are identified by PCR processes in order to detect the presence of the respective marker combination.

31. Process according to any one of claims 1 to 29, wherein haploid cells containing recombination cassettes with either first, second, third or fourth recombined DNA sequences are identified by plating

the haploid cells on media that select for the molecular linkage on the same DNA molecule of the respective marker combination.

32. Process according to claim 31, wherein haploid cells containing first recombined DNA sequences are plated on a medium that selects for molecular linkage on the same DNA molecule of the first and the fourth marker sequences.

33. Process according to claim 31, wherein haploid cells containing second recombined DNA sequences are plated on a medium that selects for molecular linkage on the same DNA molecule of the second and the third marker sequences.

34. Process according to claim 31, wherein haploid cells containing third recombined DNA sequences are plated on a medium that selects for molecular linkage on the same DNA molecule of the first and the second marker sequences.

35. Process according to claim 31, wherein haploid cells containing fourth recombined DNA sequences are plated on a medium that selects for molecular linkage on the same DNA molecule of the third and the fourth marker sequences.

36. Plasmid pMXY9, comprising adjacently the *URA3* marker gene and the *CAN1* marker gene, whereby the two marker sequences flank a polylinker sequence for inserting a DNA sequence to be recombined and whereby the two markers are flanked by targeting sequences homologous to the *BUD31-HCM1* locus on chromosome III of the S. cerevisiae genome.

37. Plasmid pMXY9 according to claim 36, wherein the polylinker sequence comprises restriction sites for the restriction enzymes *Sma*I, *Xba*I, *Pac*I and *Bgl*II.

38. Plasmid pMXY12, comprising adjacently the *TRP1* marker gene and the *CYH2* marker gene, whereby the two marker sequences flank a polylinker sequence for inserting a DNA sequence to be recombined and whereby the two markers are flanked by targeting sequences homologous to the BUD31-HCM1 locus on chromosome III of the S. cerevisiae genome.

39. Plasmid pMXY12 according to claim 38, wherein the polylinker sequence comprises restriction sites for the restriction enzymes *Sma*I, *Spe*I and *Pac*I.

40. S. cerevisiae strain MXY47, characterized in that diploid cells thereof are homozygous for the alleles *ura3-1*, *trp1-1*, *can1-100* and *cyh2R* and heterozygous for the *msh2::KanMX* mutation.

41. E. coli strain JM101, containing plasmid pMXY9.

42. E. coli strain DH5α, containing plasmid pMXY12.

43. Kit comprising at least a first container which comprises cells of S. cerevisiae strain MXY47, a second container which comprises cells of E. coli strain JM101containing plasmid pMXY9 and a third container comprising cells of E. coli strain DH5α containing plasmid pMXY12.

44. Kit comprising at least a first container comprising cells of S. cerevisiae strain MXY47, a second container comprising DNA of

plasmid pMXY9 and a third container comprising DNA of plasmid pMXY12.